

Attorney Docket No.: **WON-0003**  
Inventors: **Kwak et al.**  
Serial No.: **10/519,511**  
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#### REMARKS

Claims 1 through 3 and 7 through 9 are pending in the instant application. Claims 1 through 3 and 7 through 9 have been rejected. Claims 1, 7, 8 and 9 have been amended. Support for these amendments is provided in the specification at page 12, lines 8-18 and Example 2. No new matter is added by these amendments. Reconsideration is respectfully requested in light of these amendments and the following remarks.

#### **Rejection of Claims 1-3 and 7-9 under 35 U.S.C. 103(a)**

The rejection of claims 1-3 and 7-9 under 35 U.S.C. 103(a) as being unpatentable over Byth et al. (2000) in view of Ichinose et al. (1995) has been maintained.

Accordingly, in an earnest effort to advance the prosecution of this case, Applicants have amended the claims to state that the steps of the high throughput method comprise:

(a) culturing photomixotrophic cells in a microwell plate to which candidates for plant growth regulators were added;

(b) treating the cells of step (a) with 2,3,5-triphenyltetrazolium chloride for 4.5 to 5.5 hours;

(c) removing solutions from the microwell plate;

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(d) adding ethanol to the remaining cells and reacting for 0.5 to 2 hours;

(e) transferring the reacted solution of step (d) into a new microwell plate;

(f) measuring optical density of the microwell plate of step (e) with a high throughput screening reader; and

(g) determining the activity of candidates for plant growth regulators based on the optical density of step (f). Support for these amendments is provided in the specification at page 12, lines 8-18 and Example 2. The claims as amended highlight advantages of the instant invention which relates to the discovery by Applicants that after an hour of TTC, the extracellular formazan in medium was hardly detected and the content of intracellular formazan was decreased dose-dependently. However, after 5 hours of TTC treatment, both extracellular and intracellular formazan content were decreased dose-dependently. Further about 50% of formazan was isolated from cells, making the extracellular and intracellular formazan content almost even. See Figure 3 and teachings in the specification at page 20, 5-18 lines. The instant claimed assay is based upon the inventors' discovery that the amount of extracellular formazan after 4.5-5.5 hours of TTC treatment, reflects accurately on the amount of formed formazan inside

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cells. This modified TTC assay enables evaluation of the activity of candidates for regulators more conveniently (a troublesome cell-crushing procedure is not needed) and more rapidly (reaction time with ethanol is reduced).

In contrast to the instant claimed invention requiring treating the cells with 2,3,5-triphenyltetrazolium chloride for 4.5 to 5.5 hours and reacting the cells with ethanol for 0.5 to 2 hours, the TTC assay taught by Byth et al. involves treating the sample with TTC, centrifuging the sample, resuspending the pellet in ethanol for **16 hours** and then measuring the OD of the supernatant (see col. 1 of page 342 of Byth et al.). Ichinose et al. also teaches a method very different to that claimed wherein cells are cultured with an herbicide for **5 days** prior to measurement of chlorophyll content (see col. 2 of page 694 of Ichinose et al).

MPEP 2143 is clear; to establish a *prima facie* case of obvious, the combination of cited prior art must teach or suggest all the claim limitations. The cited combination of Byth and Ichinose et al. which neither teaches nor suggests the steps of the instant claimed assay clearly fails to meet this criterion.

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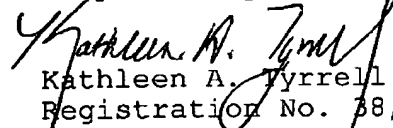
Accordingly, the cited combination of references cannot render obvious the instant claimed high throughput method for screening for a modulation in plant cell growth.

Withdrawal of this rejection under 35 U.S.C. 103(a) is therefore respectfully requested.

#### Conclusion

Applicants believe that this submission overcomes all pending rejections in this case and comprises a full and complete response to the Office Action of record. Accordingly, favorable reconsideration and subsequent allowance of the pending claims is earnestly solicited.

Respectfully submitted,

  
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